(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 31 July 2003 (31.07.2003)

PCT

(10) International Publication Number WO 2003/062409 A3

(51) International Patent Classification7:

A23K 1/165

(21) International Application Number:

PCT/DK2003/000039

(22) International Filing Date: 23 January 2003 (23.01.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: PA 2002 00130

25 January 2002 (25.01.2002) DK

- (71) Applicant (for all designated States except US): ROCHE VITAMINS AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): WU, Wenping [CN/CN]; Room 203, Er Dan Yuan in Bldg. #3, Yi Qu, Dong Li Xiao Qu, Shang Di Zone, Haidan District, 100085 Beijing (CN). PETTERSSON, Dan [SE/DK]; Poppel allé 12, DK-3540 Lynge (DK). FUGLSANG, Claus, Crone [DK/DK]; Damsted Gaard, Korshoejgaardsvej 7, DK-3670 Veksoe (DK).
- (74) Agent: NOVOZYMES A/S; Krogshøjvej 36, DK-2880 Bagsværd (DK).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

 as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

Published:

with international search report

(88) Date of publication of the international search report: 26 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERMOSTABLE ENZYME COMPOSITIONS

(57) Abstract: The present invention relates to a composition comprising at least two thermostable enzymes selected from the group consisting of endoglucanase, xylanase, phytase, protease, galactanase, mannanase, dextranase, and alpha-galactosidase. The thermostable enzymes have a melting temperature, Tm, of at least 70°C. Preferred compositions comprise a xylanase of glycoside hydrolase family 11, and an endoglucanase which is homologous to a thermostable glycoside hydrolase family 5 endoglucanase derived from Thermoascus aurantiacus. Preferred xylanases are derived from Aspergillus, Bacillus, Humicola, Thermomyces and Trichoderma. The composition is particularly useful for animal feed purposes. Optional additional components are vitamins, minerals, and anti-microbial peptides.

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A23K1/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) I PC $\,\,7\,\,\,\,\,\,$ A23 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, BIOSIS, PAJ, CHEM ABS Data, SEQUENCE SEARCH, FSTA, EPO-Internal, EMBL

Category °	ENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
Category		1 12	
X	WO 97 20920 A (FINNFEEDS INT LTD ;GENENCOR INT (US)) 12 June 1997 (1997-06-12) page 7, line 1 - line 4 page 7, line 16 - line 24 abstract; figures	1-13	
X	WO 97 22691 A (PRIMALCO LTD) 26 June 1997 (1997-06-26) page 3, line 10 - line 16 page 12, line 11 - line 15 abstract	1-13	
X	WO 98 28410 A (NOVONORDISK AS) 2 July 1998 (1998-07-02) page 17, line 27 -page 19, line 36 abstract/	1-13	

1		
X Further documents are listed in the continuation of box C.	X Patent family members are listed in annex.	
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family	
Date of the actual completion of the international search 2 July 2003	Date of mailing of the international search report 0 1 08 2003	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Carolina Palmcrantz	



Internatio oplication No
PCT/DK 03/00039

	TO DE DEL EVANT	
C.(Continua Category °	otion) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Jalegory .	Citation of document, with the control of the citation of document, with the citation of the c	
X	DATABASE WPI Section Ch, Week 200118 Derwent Publications Ltd., London, GB; Class B03, AN 1997-077310 XP002199894 -& JP 2001 057852 A (SHOWA DENKO KK), 6 March 2001 (2001-03-06) abstract	1-13
X	WO 01 90333 A (DIVERSA CORP) 29 November 2001 (2001-11-29) page 96, line 10 -page 97, line 1 page 128, line 11 - line 29 page 137 figure 3; example 1	1-13
X	BHAT M K ET AL: "Thermostable cellulase and xylanase from THERMOASCUS AURANTIACUS and their potential industrial applications." AMERICAN CHEMICAL SOCIETY SYMPOSIUM SERIES 2000, CHAPTER 12, 2000, pages 204-221, XP002245107 page 208	1,2
A	DATABASE EMBL [Online] HONG J ET AL: "Cloning of a gene encoding a thermo-stable endo-beta-1,4-glucanase from thermoascus aurantiacus and its expression in yeast." Database accession no. AY055121 XP002902919 abstract & BIOTECHNOL. LETT., vol. 25, 2003, pages 657-661, & DATABASE SWALL [Online] IFO 97 2001 to the EMBL GenBank DDBJ databases; retrieved from EBI Database accession no. Q96U15 99,403% identity in 335 aa -/	3,4

PCT/ISA/210 (continuation of second sheet) (July 1992)

---- 0 AF 2



Internati pplication No	
PCT/DK 03/00039	

		<u> </u>
C.(Continua	tion) DOCUMENTS CONSIDERED TO BE RELEVANT	In the state No.
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; October 1998 (1998-10) SALOBIR J: "Effect of xylanase alone and in combination with beta-glucanase on energy utilisation, nutrient utilisation and intestinal viscosity of broilers fed diets based on two wheat samples." Database accession no. PREV199900042049 XP002245108 abstract & ARCHIV FUER GEFLUEGELKUNDE, vol. 62, no. 5, October 1998 (1998-10), pages 209-213, ISSN: 0003-9098	1-13
A	BEDFORD M R ET AL: "Process stability and methods of detection of feed enzymes in complete diets." ENZYMES IN FARM ANIMAL NUTRITION CHAPTER 15, 2001, pages 377-386, XP002902920 the whole document	1-13

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

___ 2 ~ 5



INTERNATIONAL SEARCH REPORT



Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. X Claims Nos.: 1-3, 5-13 because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically: See FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-3, 5-13

Claims 1-2, 5, 7, 12-13 concern all compositions comprising the enzyme combinations. COmbinations of two or more thermostable enzymes are very common additives in a lot of technically different areas. In many cases it is not even possible to reveal from the prior art documents if the enzymes has a melting temperature of at least 70 degrees centigrade, since this parameter may not necessarily be disclosed.

Further, present claims 1-2, 5-13 relate to an extremely large number of possible combinations, since every group of enzymes in itself (e.g. "protease") covers many different compounds. Thus, it is considered to be impossible to perform a search for all documents that may be relevant to claims 1-2 and 5-13.

Therefore, the search has mainly been focused on animal feed compositions comprising thermostable enzyme combinations in general and especially to combinations with the endoglucanase disclosed in claim 3 (ii)(a), an allelic variant of 3 (ii)(a), and a fragment of 3(ii)(a) that has endoglucanase activity.

The wording "encoded by a nucleic acid sequence which hybridizes under low stringency conditions with " in claim 3(ii)(b), the wording " a subsequence of (i) or (ii) of at least 1000 nucleotides " in claim 3(ii)(b)(iii), the wording " a complementary strand of (iii) " in claim 3(ii)(b)(iv), and the wording " a variant of the polypeptide having amino acid sequence of SEQ ID No:2 comprising a substitution, deletion. and / or insertion of one or more amino acid " in claim 3(ii)(c) are considered to cover so many different sequences that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely to SEQ ID No:1, SEQ ID No:2 and to the variants disclosed in claim 3(ii)(a), an allelic variant of 3 (ii)(a) as disclosed in 3 (ii)(d) and a fragment of 3 (ii)(a) that has endoglucanase activity as disclosed in 3 (ii)(e).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNA NAL SEARCH REPORT

Information on patent family members

Internation No
PCT/DK 03/00039

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 9720920	A 12-06-1997	US AU AU BR CA EP JP NZ WO	5902581 A 718686 B2 1146997 A 9612118 A 2237649 A1 0865484 A1 2000501935 T 324588 A 9720920 A1	11-05-1999 20-04-2000 27-06-1997 15-06-1999 12-06-1997 23-09-1998 22-02-2000 29-11-1999 12-06-1997
WO 9722691	A 26-06-1997	AU AU EP EP WO WO US	1099597 A 1099697 A 0868506 A1 0870015 A1 9722691 A1 9722692 A1 6228629 B1 5922579 A	14-07-1997 14-07-1997 07-10-1998 14-10-1998 26-06-1997 26-06-1997 08-05-2001 13-07-1999
WO 9828410	A 02-07-1998	AU BR WO EP JP TR US	7873598 A 9713780 A 9828410 A1 0956348 A1 2001507221 T 9901393 T2 6043075 A	17-07-1998 21-03-2000 02-07-1998 17-11-1999 05-06-2001 23-08-1999 28-03-2000
JP 2001057852	A 06-03-2001	WO	9700020 A1	03-01-1997
WO 0190333	A 29-11-2001	AU CA EP WO US US	6345601 A 2411199 A1 1301592 A2 0190333 A2 2003049815 A1 2003103958 A1 2002136754 A1	03-12-2001 29-11-2001 16-04-2003 29-11-2001 13-03-2003 05-06-2003 26-09-2002